European Patent Office
Erhardtstrasse 27
D-80331 München
GERMANY

Subject: Re:	Opposition to European Patent EP 0 120 694 B1 Appl. No.: 84 301 996.9
Patent Owner:	Celltech Limited 244-250 Bath Road Slough Berkshire SL1 4DY (GB)
Title:	PROCESSES FOR THE PRODUCTION OF MULTICHAIN POLYPEPTIDES OR PROTEINS

- and -

Subject: Re:	Opposition to European Patent EP 0 125 023 B1 Appl. No.: 84 302 368.0
Patent Owner:	Genentech, Inc. South San Francisco, California United States of America
Title:	RECOMBINANT IMMUNOGLOBULIN PREPARATIONS, METHODS FOR THEIR PREPARATION, DNA SEQUENCES, EXPRESSION VECTORS AND RECOMBINANT HOST CELLS THEREFOR

DECLARATION OF ROBERT G. HAMILTON

I, Robert G. Hamilton, declare:

1. I am currently an Associate Professor of Medicine at the Johns Hopkins University School of Medicine. In 1983 I was an Assistant Professor of Medicine at the Johns Hopkins University School of Medicine and Director of the Johns Hopkins University Dermatology, Allergy and Clinical Immunology Reference Laboratory. My federally-licensed clinical laboratory performs routine diagnostic allergy and immunology testing. As such, I consider myself an expert on antibodies. A copy of my curriculum vitae is attached as Exhibit A.

2. On 17 March 1983 I attended the Mallinkrodt Award Lecture given by Dr. Marc Shulman at the 9th Annual Meeting of the Clinical Ligand Assay Society. As Chairman of the session that included the Mallinkrodt Award Lecture, I speak authoritatively as to content of the lecture and the absence of restrictions on use of information presented by Dr.

Shulman. The lecture, "Monoclonal Antibodies: The Prospects for Serious Engineering" was open to the public and there were no instructions to keep the content of the lecture confidential. As stated in Dr. Shulman's declaration, the lecture was attended by approximately 100 people.

3. I have recently read the Declaration of Dr. Marc Shulman made on 21 May 1994 regarding this lecture and filed in the European Patent Office in connection with the Opposition to European Patent EP 0 125 023 B1, and I agree with what he says.

4. I have a clear recollection of this lecture. After reviewing basic knowledge of immunoglobulins, corresponding genes, and hybridoma technology, Dr. Shulman went on to introduce the concept of using recombinant DNA techniques to produce (engineer) heterologous (human/mouse) antibodies.

5. Dr. Shulman discussed the preparation of human/mouse chimeric antibodies by recombinant DNA techniques. He described how both heavy and light immunoglobulin chains could be expressed and properly assembled in a single host cell. The cell lines that were mentioned as host cells for chimeric antibody production were lymphoid cells, which did not produce endogenous immunoglobulin light or heavy chains, for example SP2/0.

6. Dr. Shulman discussed immunoglobulin gene arrangement and rearrangement at the VDJ region, which occurs in nature to produce a functional antibody encoding gene. He indicated that analytical techniques were being used in his laboratory to produce chimeric antibodies, in a single host cell, having a constant region derived from one species, usually man, and a variable region derived from a second species, usually mouse. Dr. Shulman's lecture in combination with the detailed knowledge of immunoglobulin gene structure and function provided the information necessary for understanding how to produce chimeric antibodies in a single recombinant host cell.

7. Dr. Shulman proposed mimicking nature's processes by cutting the immunoglobulin gene in a non-coding region between the rearranged VDJ region and the C region. A second cut in the C region below the hinge allows excision of this DNA fragment from its vector site. An equivalent, but heterologous DNA fragment encoding a C region was ligated between the two cut sites. From this, it was apparent to me how one would combine a

variable region gene fragment from a mouse source with a constant region fragment from a human source to produce DNA encoding an active chimeric gene in one host cell.

8. Anyone listening to Dr. Shulman's entire lecture would have come away with similar ideas for using these new techniques to develop diagnostically useful immunologic reagents and therapeutic antibodies with reduced immunogenicity in man.

9. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and beliefs are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

May 16, 1996
Date

Robert G. Hamilton
Robert G. Hamilton, Ph.D., D.ABMLI
Associate Professor of Medicine

Melinda L. Welsh
Melinda L. Welsh, Baltimore County Notary
My Commission expires 7/22/98

Exhibit A

050696

CURRICULUM VITAE
Robert G. Hamilton

Current Appointment:
Associate Professor of Medicine
Johns Hopkins University School of Medicine, Baltimore, MD

Personal Data:
Date of Birth: April 8, 1951
Citizenship: United States of America
Military Service: Lieutenant, US Navy, MSC, National Naval Medical Center, Bethesda, MD, Radiopharmacist, Honorable Discharge, 03-1977

Education and Training: (Chronological Order)

1967-1968: Liceo Scientifico, Varese, Italy

1969-1973: University of Michigan, Ann Arbor, MI
B.S. Chemistry, Biology (1972)
M.S. Radiological Physics (1973)

1971-1972: University of Florence, Italy
(Junior year abroad with Smith College, Northampton MA)

1974: Brookhaven National Laboratory, Long Island, NY
Fellowship in reactor, linear accelerator and medical physics

1975: National Naval Medical Center, Diplomate, Officer's Program in Nuclear Medicine and Radioisotopes

1977-1980: Johns Hopkins University, Baltimore, MD
Ph.D., Radiological Sciences and Immunology (1980)

1980: Postdoctoral Fellowship, Dr. Rosalyn Yalow, Nobel Laureate, VA Medical Center, Bronx, NY

Certification:

1979: Diplomate, *American Board of Science in Nuclear Medicine*
American Society of Nuclear Medicine, Laboratory Speciality

1982: Diplomate, *American Board of Science in Nuclear Medicine*
American Society of Nuclear Medicine, Radiochemistry and Radiopharmacy Speciality

1992: Diplomate, *American Board of Medical Laboratory Immunology*
American Society for Microbiology

Professional Experience:

1980-1981: Veterans Administration Medical Center, Bronx, NY
Department of Radiological Physics, Medical and Health Physics for Nuclear Medicine, Diagnostic Radiology and Therapeutic Radiology

1980-1981: Assistant Professor of Radiology
Albert Einstein College of Medicine, Bronx, NY

1981-1984: Assistant Professor of Medicine
Medicine, Johns Hopkins University School of Medicine, Baltimore, MD

1981-1984: Director, Dermatology-Allergy Clinical Immunology
(DACI) Reference Laboratory Johns Hopkins University School of Medicine

1984-1990: Assistant Professor of Medicine
Rheumatology, University of Texas School of Medicine, Houston, TX

1984-1988: Assistant Professor of Biomedical Sciences
University of Texas, Graduate School of Biomedical Sciences, Houston TX

1990-Present: Associate Professor of Medicine
Medicine, Johns Hopkins University School of Medicine, Baltimore, MD

1990-Present: Director, Dermatology-Allergy Clinical Immunology (DACI)
Reference Laboratory Johns Hopkins University School of Medicine

Professional Activities:

1985-1987: American Association for Clinical Chemistry
Chairman, Division of Diagnostic and Clinical Immunology

1986-1988: Food and Drug Administration, Washington DC
Gasteroentology and Hematology Advisory Committee Devices Review Pane

1987-1990: American Association for Clinical Chemistry
Oak Ridge Conference Scientific Committee Member

1989-Present: International Union of Immunological Societies
Standardization Committee, Subcommittee of Human Immunoglobulin
Classes/Subclasses, Member

1989-Present: Italian Clinical Chemistry Society
ATB European Oak Ridge Conference Scientific Committee

1992-Present: College of American Pathologists (CAP)
Clinical and Diagnostic Immunology Committee-Member
Coordinator of SE-Diagnostic Allergy Inter-Laboratory Quality Control
Survey Program for United States

1993-Present: National Committee on Clinical Laboratory Standards
(NCCLS) Co-Chair, Diagnostic Allergy Subcommittee Diagnostic
Immunology Committee

1994-Present: American Academy of Asthma, Allergy and Immunology
(AAAAI), In vitro Committee, Chairman

Professional Societies

American Association of Immunologists (Present)
American Academy of Allergy, Asthma and Immunology (Present)
American College of Allergy and Immunology (Present)
Association of Medical Laboratory Immunologists (Present)
American Society for Nuclear Medicine (Past)

American Association of Clinical Chemistry (Past)
Clinical Ligand Assay Society (Past)
American Health Physics Society (Past)

Editorial Activities:

1980-1994: *Journal of Clinical Immunoassay*: Editorial Board
1988-present: *Journal of Immunoassay*: Editorial Board
1990-present *Journal of Clinical Biochemistry*: Reviewer
1992-present *Journal of Allergy and Clinical Immunology*: Reviewer
1992-present *Clinical Chemistry*: Reviewer
1992-present *Immunology Letters*: Reviewer
1992-present *Clinical and Diagnostic Immunology*: Editorial Board
1996-present *Annals of Allergy, Asthma and Immunology*, Editorial Board
1996-present *J. Immunology*, Reviewer

Honors, Awards:

1976 Armed Forces Radiobiology Research Institute (AFRRI) Award
Best paper, Eastern Society of Nuclear Medicine

1978-1980 Predoctoral Fellowship, Johns Hopkins University

1979- Mallinckrodt Graduate Student Research Fellowship
Society of Nuclear Medicine, Education and Research Foundation

1980- Berson-Yalow Award
American Society of Nuclear Medicine

Institutional Administrative Appointments: (none)

Teaching:

I. Johns Hopkins University Advisees (Pre and Post doctoral)

Post Doctoral: Yong Tsai, MD (Clinical Immunology)
Post Doctoral: Eric Shampaine, MD (Clinical Immunology)
Post Doctoral: Stokes Peebles, M.D. (Clinical Immunology)
Post Doctoral: Michael Wein, M.D. (Clinical Immunology)
Post Doctoral: Robert Zeldin, M.D. (Clinical Immunology)
Post Doctoral: Jorg Kleine-Tebbe, M.D. (Clinical Immunology)
Doctoral Student: Rodger Lewis (Environmental Health Science)
Post Doctoral: Michael Goldman, M.D. (Clinical Immunology)
Post Doctoral: Kenneth Kurtz, M.D. (Clinical Immunology)

II. Thesis Committee

Paul Kussie (Ph.D. Immunology-Houston Texas)
Rodger Lewis (Ph.D. Environmental Health Science, Johns Hopkins Univ)

III. Classroom Instruction with Course Titles

Immunological methods (Johns Hopkins Hospital)

Publications:

I. Peer Reviewed Articles

1. **Hamilton RG**, PO Alderson, JF Harwig, BA Siegel. Splenic imaging with Tc-99m labeled erythrocytes: a comparative study of cell damaging methods *J Nucl Med 17*:1039-43, 1976.

2. **Hamilton RG**, PO Alderson, PA McIntyre. Technetium-99m phytate as a bone marrow imaging agent: biodistribution studies in animals. *J Nucl Med 19*:563-5, 1977.

3. **Hamilton RG**, PO Alderson. A comparative evaluation of techniques for rapid and efficient *in vivo* labeling of red blood cells with Tc-99m pertechnetate. *J Nucl Med 18*:1008-11, 1977.

4. Bradley WP, PO Alderson, WC Eckelman, **RG Hamilton**, JF Weiss. Decreased tumor uptake of Gallium-67 in animals after whole body irradiation. *J Nucl Med 19*:204-9, 1978.

5. **Hamilton RG**, RA Walker. Labeling of endotoxin lipopolysaccharide with technetium-99m pertechnetate. *Experimentia 34*(Supppl 5), 1978.

6. **Hamilton RG**, A Kagey-Sobotka, NF Adkinson Jr. Solid phase radioimmunoassay for quantitation of antigen-specific IgG using I-125 labeled Protein A from *Staphylococcus aureus*. *J Immunol 122*:1073-1079, 1979.

7. **Hamilton RG**, NF Adkinson Jr. Quantitation of antigen-specific IgG in human serum. I. Standardization by a *Staphococcal aureus* solid phase radioimmunoassay elution technique.*J Immunol 124*:2966-71, 1980.

8. **Hamilton RG**, M Rendell, NF Adkinson Jr. Serological analysis of human IgG and IgE anti-insulin antibodies using solid phase radioimmunoassays. *J Lab Clin Med 96*:1023-36, 1980.

9. **Hamilton RG**, NF Adkinson Jr. Quantification of antigen-specific IgG in human serum. II. Comparison of radioimmunoprecipitation and solid phase RIA techniques for the measurement of IgG specific for a complex antigen mixture (yellow jacket venom). *J Allergy Clin Immunol 67*:14-21, 1981.

10. Verias R, **RG Hamilton**, MP Grissom RF Kiepffer, JF Vandergrift. Evaluation of Indium-111 colloid for radionuclide imaging of the abdominal lymph nodes. *Nucl Med Comm* 2:37-42, 1981.

11. **Hamilton RG**, R Hussain EA Ottesen, NF Adkinson Jr. The quantification of parasite-specific human immunoglobulins: comparison of RIA and ELISA methods. *J Immunol Meth 44*:101-14, 1981.

12. Rendell M, **RG Hamilton** HM Drew, NF Adkinson Jr. Exacerbation of diabetes mellitus by antibodies to exogenous insulin. *Am J Med Sci 282*:18-26, 1981.

13. Renie A, **RG Hamilton**, NF Adkinson Jr, MS Rendell. A case of hyper-labile diabetes accompanied by insulin resistance. *Clin Chem 27*:1463-64, 1981.

14. Hussain R, **RG Hamilton**, V Kumaraswami, NF Adkinson Jr, EA Ottesen. IgE responses in human filariasis. I. Quantification of filarial-specific IgE. *J Immunol 127*:1623-29, 1981.

15. Button TM, **RG Hamilton**. Activated charcoal filter counting for radioiodine effluent concentration determined in protein iodinations.*Health Physics 43*:853-9, 1982.

16. **Hamilton RG**, NF Adkinson Jr. Serum IgE antibodies by the radioallergosorbent test (RAST): diagnosis of IgE mediated diseases. *Immunopath 6*:1-9, 1982.

17. Gentlesk MJ, **RG Hamilton**, NF Adkinson Jr., HC Mansmann. Assessment of venom-specific IgG antibody in patients treated for Hymenoptera venom sensitivity. *Int Arch Allergy Appl Immunol 71*:233-40, 1983.

18. **Hamilton RG**, A Scott, R D'Antonio, DA Levy, NF Adkinson Jr. *Dirofilaria imitis*: Performance and standardization of specific antibody immunoassays for Filariasis. *Experimental Parasitol 56*:298-312, 1983.

19. **Hamilton RG**, R Hussain, E Alexander, NF Adkinson Jr. Limitations of the radioimmunoprecipitation polyethylene glycol assay (RIPEGA) for detection of filarial antigens in serum. *J Immunol Meth 68*:349-66, 1984.

20. **Hamilton RG**, A Scott. Immunoradiometric assay for quantitation of *Dirofilaria immitis* antigen in dogs with heartworm infections. *Am J Vet Res 45*:1-7, 1984.

21. Hussain R, **Hamilton RG**, EA Ottesen. Immunoradiometric assays detection of filarial antigens in human serum. *J Immunol 133*:2237-42, 1984.

22. Ditu M, JM Waud, **RG Hamilton**, HN Wagner Jr. Obtention automatisee des profils de precision des dosages radioimmunologiques de T4 a' l'aide d'une calculatrice de poche de type HP-41CV. *Annal d'endocrinol (Paris) 45*:393-6, 1984.

23. **Hamilton RG**, NF Adkinson Jr. Hypersensitivity reactions in hemodialysis: Mechanisms of acute allergic reactions. *Artifical Organs 8*:311-7, 1984.

24. Reddy MVR, **RG Hamilton**, BC Harinath. Detection of filarial antigen in the urine of humans with *Wuchereria bancrofti* infection by immunoradiometric assay. *Indian J Exp Biol 22*:515-519, 1984.

25. **Hamilton RG**, NF Adkinson Jr. Naturally-occurring carbohydrate antibodies: Interference in solid phase immunoassays. *J Immunol Meth 77*:95-108, 1985.

26. **Hamilton RG**. Application of immunoassay methods in the serodiagnosis of human Filariasis. *Rev Infect Dis 7*:837-43, 1985.

27. **Hamilton RG**, M April, E Wagner, A Kagey-Sobotka, J Winkelstein, NF Adkinson Jr, E Bleeker. Diethylcarbamazine reactions in *Dirofilaria immitis* infected dogs:

Relationship between physiology, antigen, antibody, complement, and vasoactive substances. *Experimental Parasitol 61*:405-20, 1986.

28. **Hamilton RG**, GR Mintz, MK Gelbard. Humoral immune response in Peyronie's disease patients receiving clostridial collagenase therapy. *J Urol 135*:641-7, 1986.

29. Paranjape RS, R Hussain, TB Nutman, **RG Hamilton**, EA Ottesen. Identification of circulating parasite antigen in patients with Bancroftian filariasis. *Clin exp Immunol 63*:508-16, 1986.

30. Parkhe KA, GBKS Prasad, A Das, BC Harinath, M Roebber, **RG Hamilton**. Disc/stick ELISA for diagnosis of Bancroftian filariasis. *Indian J Exp Biol 24*:437-9, 1986.

31. **Hamilton RG**, NF Adkinson Jr. Laboratory methods in the quantitation of Hymenoptera venom-specific IgE and IgG human antibodies. *Folia Allergol Immunol Clin 33*:31-52, 1986.

32. **Hamilton RG**, M Roebber, CB Reimer, SL Rodkey. Isoelectric focusing patterns of mouse monoclonal antibodies to the four human IgG subclasses. *Electrophoresis 9*:127-134, 1987.

33. Prasad GBK, BC Harinath, **RG Hamilton**. Analysis of paired serum, urine and filter paper blood specimens for the presence of filarial antigen by immunoradiometric assay. *J Immunoassay 9*:351-365, 1987.

34. **Hamilton RG**, M Roebber, CB Reimer, SL Rodkey. Quality control of murine monoclonal antibodies using isoelectric focusing immunoblot analysis. *Hybridoma 6*:204-217, 1987.

35. **Hamilton RG**. Human IgG subclass measurements in the clinical laboratory. *Clin Chem 33*:1070-5, 1987.

36. Jones CC, **RG Hamilton**, RE Jordon. Subclass distribution of human IgG autoantibodies in pemphigus. *J Clin Immunol 8*:43-9, 1988.

37. **Hamilton RG**, JB Harley, M Reichlin, M Roebber, MC Hockberg, WB Bias, FC Arnett. Two Ro(SSA-A) autoantibody responses in systemic lupus erythematosus: Correlation of HLA DR/DQ specificities with quantitative expression of Ro(SS-A) autoantibody. *Arth Rheum 31*:496-506, 1988.

38. **Hamilton RG**, H Baum, NF Adkinson Jr. Immunoassay characterization of human IgG subclass immune responses in patients receiving insulin therapy and ragweed immunotherapy. *J Clin Immunoassay 11*:24-30, 1988.

39. **Hamilton RG**, RW Wilson, T Spillman, M Roebber. Monoclonal antibody-based immunoenzymetric assays for quantification of human IgG and its four subclasses. *J Immunoassay 9*:275-96, 1988.

40. Arnett FC, **RG Hamilton**, M Roebber, JB Harley, M Reichlin. Increased frequency of Sm and nRNP autoantibodies in American Blacks compared to Whites with systemic lupus erythematosus. *J Rheumatol 15*:1773-6, 1988.

41. Fasullo FJ Jr, HA Fritsche Jr, F Liu, **RG Hamilton**. IgG heavy chain subclass typing of myeloma paraprotein by isoelectric focusing (IEF) and immunoblot analysis. *Clin Chem 35*:364-8, 1989.

42. **Hamilton RG.** Monoclonal antibodies in the diagnosis and therapy of human diseases. *Ann Biol clin 47*:575-81, 1989.

43. **Hamilton RG.** Autoantibodies to immunoglobulins: Rheumatoid factor interference in immunological assays. *Monogr Allergy 26*:27-44, 1989.

44. Arnett FC, **RG Hamilton**, JD Revelle, WB Bias, JB Harley, M Reichlin. Genetic studies of Ro(SS-A) and La(SS-B) autoantibodies detected by ELISA in families with systemic lupus erythematosus and primary Sjogren's syndrome. *Arth Rheum 32*:413-9, 1989.

45. Musher DM, MJ Luchi, DA Watson, **RG Hamilton**, R Baughn. Pneumococcal polysaccharide vaccine in young adults and older bronchitics: Determination of IgG responses by ELISA and the effect of adsorption of serum with non-type specific cell wall polysaccharide. *J Infect Dis 161*:728-35, 1990.

46. **Hamilton RG.** Engineered human antibodies as immunologic quality control reagents. *Ann Biol Clin 48*:473-7, 1990.

47. Chang TW, FM Davis, NC Sun, CRY Sun, DW MacGlashan Jr, **RG Hamilton**. Monoclonal antibodies specific for human IgE producing B-cells: a potential therapeutic for IgE mediated allergic diseases. *Biotech 9*:122-126, 1990.

48. Moulds JM, FC Arnett, CGG Giles, **RG Hamilton**. A novel immunoassay for the quantitation of human C4 gene products. *Complement Inflammation 7*:95-9, 1990.

49. **Hamilton RG.** Allergy testing. *Current Opin Immunol* 2: 558-64, 1990.

50. **Hamilton RG.** Application of engineered chimeric antibodies to the calibration of human antibody standards. *Ann Biol Clin. 49*:242-248, 1991.

51. Keating MU, Kagey Sobotka A, **Hamilton RG**, Yunginger JW. Clinical and immunological follow-up of patients who discontinue venom immunotherapy. *J Allergy Clin Immunol 88*:339-48, 1991.

52. Herrman DJ, **RG Hamilton**, T Barington CE Frash, G Arakere, O Makela, LA Mitchell, J Nael, GT Rijkers, B Zegers, B Danve, JI Ward, CS Brown. Quantitation of human IgG subclass antibodies to *Haemophilus influenzae* Type b capsular polysaccharide: Results of an international collaborative study using enzyme immunoassay methodology. *J Imunol Meth 148*:101-14, 1992.

53. Lichtenstein LM, A Kagey-Sobotka, JM White, **RG Hamilton**. Anti-human IgG causes basophil histamine release by acting on IgG-IgE complexes bound to IgE receptors. *J Immunol 148*:3929-36, 1992.

54. DiPiro JT, **RG Hamilton**, TR Howdieshell, NF Adkinson Jr., AR Mansberger Jr. Total IgE in plasma is elevated after traumatic injury and is associated with sepsis. *Ann Surgery 215*:460-6, 1992.

55. Wilson ME, **RG Hamilton**. IgG subclass response of localized juvenile periodontitis subjects to *Actinobacillus actinomycetemcomitans* Y4 lipopolysaccharide. *Infection Immunity60*:1806-12, 1992.

56. Golden DBK, ID Lawrence ID, **RG Hamilton**, A Kagey Sobotka, MD Valentine, LM Lichtenstein. Clinical correlation of the venom-specific IgG antibody level during maintenance venom immunotherapy. *J Allergy Clin Immunol 90*:386-93, 1992.

57. DiPiro JT, **RG Hamilton**, JP Wei. Novel antibody drug products. *Am J. Surg* 164:77-84, 1992.

58. **Hamilton RG**, SM Morrison. Epitope mapping of human IgG specific murine monoclonal antibodies with domain-switched, deleted and point-mutated chimeric antibodies. *J Immunol Meth 158*:107-22, 1993.

59. Batard T, B Basuyaux, P Lambin, C Bremard-Oury, **RG Hamilton**, B David, G Peltre. Isotypic analysis of grass pollen-specific immunoglobulins in human plasma. I. Specialization of certain classes and subclasses in the immune response. *Intern Arch Allergy Immunol* 10:68-73, 1993.

60. Kuppers RC, IM Outschoorn, **RG Hamilton**, CL Burek, NR Rose. Quantitative measurement of human thyroglobulin-specific antibodies by use of a sensitive enzyme-linked immunoassay. *Clin Immunol Immunopath 67*:1-10, 1993.

61. **Hamilton RG**, JA Wisenauer, DB Golden, MD Valentine, NF Adkinson Jr. Selection of Hymenoptera venoms for immunotherapy based on patient's IgE antibody crossreactivity. *J Allergy Clin Immunol* 92:651-9, 1993.

62. **Hamilton RG.** Molecular Engineering: Applications to the Clinical Laboratory. *Clin Chem* 39:1988-92, 1993. (Oak Ridge Conference Manuscript)

63. Naclerio RM, NF Adkinson Jr., PS Creticos, FM Baroody, **RG Hamilton**, LM Lichtenstein, PS Norman. Intra-nasal steroids inhibit seasonal increases in ragweed specific IgE. *J Allergy Clin. Immunol 92*:717-21, 1993.

64. DiPiro, JT, NF Adkinson Jr., **RG Hamilton**. Facilitation of penicillin haptenation to serum proteins. *Antimicrobial Agents Chemotherapy* 37:1463-7, 1993.

65. Fifield, R, **RG Hamilton**. Inter-laboratory "external" quality assessment programs for the diagnostic allergy laboratory. *J Clin Immunoassay* 16:144, 1993.

66. Batard T., Basuyaux, A Laroze, P Lambin C Bremard Oury, P Aucouturier, **RG Hamilton**, B David, G Peltre. Isotypic analysis of grass pollen-specific immunoglobulins

in human plasma II. Quantification of the IgE, IgM IgA class and the IgG subclass antibodies. *Intern Arch Allergy Immunol* 102:279-287, 1993.

67. Dipiro JT, **RG Hamilton**, TR Howdieshell, NF Adkinson Jr., AR Mansberger Jr. Lipopolysaccharide-reactive immunoglobulin E is associated with lower mortality and organ failure in traumatically injured patients. *Clin Diag Immunol* XX:295-298, 1994.

68. **Hamilton RG**, NF Adkinson, Jr. Serological assays for antigens and antibodies. in. *Immunology and Allergy Clinics 14*:351-369, 1994.

69. Tomazic VJ, EL Shampaine, A Lamana, TJ Withrow, NF Adkinson Jr., **RG Hamilton**. Cornstarch powder on latex products is an allergen carrier. *J Allergy Clin Immunol 93*:751-758, 1994.

70. **Hamilton RG**, BL Charous, NF Adkinson Jr., JW Yunginger. Serological methods in the laboratory diagnosis of latex rubber allergy: Study of non-ammoniated, ammoniated and glove extracts as allergen reagents sources. *J Lab Clin Med 123*:594-604, 1994.

71. Charous BL, **RG Hamilton**, JW Yunginger. Occupational latex exposure: characteristics of contact and systemic reactions in 47 workers. *J Allergy Clin Immunol 94*:12-18, 1994.

72. **Hamilton RG.** The Clinical Immunology Laboratory of the Future. *Clin Chem* 40:2186-2192, 1994. (A.O. Beckman Conference Manuscript)

73. Peebles S, M Liu, LM Lichtenstein, **RG Hamilton**. IgA, IgG and IgM quantification in bronchoalveolar lavage fluids from allergic rhinitics, allergic asthmatics and normal subjects by monoclonal antibody-based immunoenzymetric assays. *J. Immunol Meth. 179:77-86, 1995.*

74. Wilson ME, **RG Hamilton**. IgG2 antibodies promote neutrophil killing of *Actinobacillus actinomycetemcomitans. Infection Immunity* 63:1070-1075, 1995.

75. Kleine-Tebbe J, **RG Hamilton**, M. Roebber, LM Lichtenstein, SM MacDonald. Purification of immunoglobulin E (IgE) antibodies from sera of high IgE titers. *J. Immunol Meth. 179:153-164, 1995.*

76. Tomazic, VJ, TJ Withrow, **RG. Hamilton**. Characterization of allergen(s) in latex protein extracts. *J Allergy Clin Immunol* 96:635-642, 1995.

77. Wilson ME, **RG Hamilton**. IgG subclass response on juvenile periodontitis subjects to principal outer membrane proteins of *Actinobacillus actinomycetemcomitans. Infection Immunity* 63:1062-1069, 1995.

78. DiPiró JT, TR Howdieshell, JK Goddard, DB Callaway, **RG Hamilton**, AR Mansberger. Association of interleukin 4 plasma levels with traumatic injury and clinical course. *Arch Surg*. 130:1159-1163, 1995.

79. Sarpong SB, **RG Hamilton**, PA Eggleston, NF Adkinson Jr., Socieoeconomic status and race as risk factors for cockroach allergen exposure and sensitization in asthmatic children. *J Allergy Clin Immunol* 97:xx-xxx, 1996.

80. Nyhan DP, EL Shampaine, CA Hirshman, **RG Hamilton**, SM Frank, WA Baumgartner, NF Adkinson Jr. Single doses of intravenous protamine results in the formation of protamine specific IgE and IgG antibodies. *J Allergy Clin. Immunol* 97:991-7, 1996.

81. Kaczmarek, RG, BG Silverman, TP Gross, **RG Hamilton**, E Kessler, JT Arrowsmith-Lowe, RM Moore Jr. Rubber latex-specific IgE antibodies among emergency room workers: Results of a multi-center prevalence study. *Ann Allergy Asthma and Immunol*. 76:51-56, 1996.

82. DiPiro JT, TR Howdieshell, **RG Hamilton**, NF Adkinson Jr, R Mansberger Jr. Increased plasma IgE in patients with sepsis after tramatic injury. J Allergy Clin Immunol 97:135-6, 1996.

83. Ahmed F, MC Steinhoff, MC Rodriguez-Barrados, **RG Hamilton**, DM Musher, KE Nelson. Effect of human immunodeficiency virus Type 1 infection on the immunogenicity of glycoprotein conjugate pneumococcal vaccine: results from a randomized trial. *J Infect Dis* 173:83-90, 1996.

84. Yeang HY, E Sunderasan, S Hamzah, S Hamid, **RG Hamilton**, MJ Cardosa. The 14.6 kd (REF, *Hev b* I) and 24 kd (*Hev b* III) rubber particle proteins are recognized by IgE from spina bifida patients with latex allergy. *J Allergy Clin Immunol* (in press).

85. Madore DV, P Anderson, BD Baxter, GM Carlone, KM Edwards, **RG Hamilton**. Inter-laboratory study evaluating quantitation of antibodies to *Haemophilus influenzae* type b polysaccharide by ELISA. *Clinical and Diagnostic Laboratory Immunology 3*:84-88, 1996.

86. **Hamilton RG**. Diagnosis of natural rubber latex allergy. *Clin Immun. Newslet.* 16:28-32, 1996.

87. Vaswani SK, **RG Hamilton**, MD Valentine, NF Adkinson Jr. Psyllium laxative induced anaphylaxis, asthma and rhinitis. Allergy (in press).

88. Mestecky J. **RG. Hamilton**, CGM Magnusson, R Jefferis, JP Vaerman, M Goodall, GG de Lange, I Moro, P Aucouturier, J Radl, C Cambiaso, C Silvain, JL Preud'homme, K Kusama, GM Carlone, J Biewenga, K Kobayashi, CB Reimer. Evaluation of monoclonal antibodies with specificity for human IgA, IgA subclasses and allotypes and secretory component. Results of an IUIS/WHO collaborative study. *J. Immunol. Meth.* (in press).

89. Naclerio RM, NF Adkinson Jr, B Moylan, FM Baroody, D Proud, A Kagey-Sobotka, LM Lichtenstein, **RG Hamilton**. Nasal provocation with allergen induces a secondary serum IgE antibody response. *J Allergy Clin Immunol* (in press).

90. **Hamilton RG**, NF Adkinson Jr. Natural rubber latex diagnostic skin testing reagents. Comparative performance of non-ammoniated latex, ammoniated latex and latex rubber glove extracts. *J. Allergy Clin Immunol.* (in press)

91. Siler DJ, K Cornish, **RG Hamilton.** Absence of cross-reactivity of IgE antibodies from *Hevea brasiliensis* latex allergic subjects with a new source of natural rubber latex from guayule (*Parthenium argentatum*). *J. Allergy Clin. Immunol.* (in press).

92. Arshad SH, **RG Hamilton** and NF Adkinson Jr. Repeated aeroallergen exposure to small doses of allergen in sensitized individuals - a model for chronic allergic asthma. *J. Allergy Clin. Immunol.* (submitted)

93. Baskar S, **RG Hamilton,** PS Norman, AA Ansari. Grass immunotherapy induces inhibition of allergen-specific human PBMC proliferation. Restoration by interleukin-1. *Int. Arch. Allergy Immunol.* (submitted).

94. Peebles RS, MC Liu, LM Lichtenstein, NF Adkinson Jr., **RG Hamilton.** Changes in bronchoalveolar lavage fluid secretory IgA and total IgA concentrations as a result of segmental antigen challenge. *J Allergy Clin Immunol* (submitted).

95. MacGlashan DW, BS Bochner, DC Adelman, PM Jardiu, A Toias, J McKenzie-White, SA Sterbinsky, **RG. Hamilton,** LM Lichtenstein. Down regulation of Fc-epsilon receptor I expression on human basophils during anti-IgE antibody therapy. *J. Allergy Clin. Immunol.* (submitted)

II. Non-Peer-Reviewed Articles

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The European Patent Office
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Opposition to European Patent 0 120 694 B1

Application No:	84301996.9
Patent Owner:	Celltech Therapeutics Limited Slough, Berkshire SL1 4DY (GB)
Title:	Processes for the Production of Multichain Polypeptides or Proteins

Declaration of Vernon T. Oi

I, Vernon T. Oi, declare:

1. I have been working in the fields of molecular immunology and antibody engineering since 1977. I am currently a senior researcher in Genetics at the Beckman Center of Stanford University, Palo Alto, California, and the President of ScienceXchange, a company formed to disseminate scientific information over the Internet. My *Curriculum Vitae* is attached hereto as Appendix A.

2. I participated in one of the first research efforts to obtain expression of functional immunoglobulin genes in mammalian cells: Proc. Natl. Acad. Sci. 80, 825-829 (Feb. 1983) (cited in the Opposition papers as "Oi"; hereinafter "1983 PNAS paper"). Beginning in 1980, our research group at Stanford University, among others, was conducting research on the regulation and expression of immunoglobulin genes; the biosynthesis, chain assembly and secretion of immunoglobulin heavy and light chains; and

the structure-function correlates of antibody molecules. In 1984, our Stanford research group was the first to publish in the scientific literature that we had obtained the expression and secretion in mammalian lymphoid cells of chimeric mouse variable/human constant region antibody molecules of defined antigen-binding specificity: Proc. Natl. Acad. Sci. 81, 6851-6855 (Nov. 1984) ("Morrison II" in the Opposition papers). From 1983 to 1992, I conducted research and development in antibody engineering at Becton Dickinson Immunocytometry Systems, rising from Senior Scientist to Vice President for Research.

3. I have reviewed EP 0 120 694 B1 ("the Boss Patent"), the priority application GB 8308235 that was filed on March 25, 1983 ("the priority document"), and the Celltech attorney's comments dated June 6, 1995, in response to the Oppositions and various third party observations filed in the European Patent Office (hereinafter "Celltech Response"). In this Declaration, I provide my observations on the priority document and Boss Patent disclosures relating to the sufficiency of their teaching in producing intact, functional heterologous immunoglobulin molecules in mammalian host cells. I also comment upon certain statements made in the Celltech Response.

4. In March 1983, a skilled person would not have been able to apply the procedures disclosed in the priority document or the Boss Patent to produce immunoglobulin molecules in mammalian cells. Both disclosures teach only the use of cDNA for immunoglobulins in the host cells disclosed. Due to the state of the art in 1983, it would not have been possible for the skilled person to construct cDNA immunoglobulin genes which could be expressed in a mammalian host cell system. A skilled person in March 1983 would not therefore have been able to follow the instructions of the priority document or of the Boss Patent to obtain expression of intact, functional immunoglobulin molecules after transformation of mammalian host cells with heterologous cDNA sequences.

5. The priority document and the Boss patent disclose the use of plasmids containing cDNA encoding immunoglobulin light and heavy chains. The cDNA-containing plasmids would not have contained the proper regulatory elements for

immunoglobulin gene expression in mammalian cells. These elements had not been characterised at the time. Without such proper regulatory elements, transcription of cDNA to produce immunoglobulins in mammalian cell systems was not possible or feasible.

6. Because of the unknowns surrounding immunoglobulin gene regulation and expression, skilled persons in 1983 used genomic immunoglobulin genes, instead of cDNA, to obtain stable expression of heterologous light chains in lymphoid cells. The genomic genes were known to contain all of the necessary regulatory elements even though location, mode of operation and function were not completely known. The first reported successful results showing expression of immunoglobulin molecules all used genomic clones: see Rice and Baltimore, Proc. Natl. Acad. Sci. 79, 7862-7865, 1982 ("Rice" in the Opposition papers); our 1983 PNAS paper ("Oi") and Ochi *et al*, Nature 302, 340-342, 1983 ("Ochi I" in the Opposition papers).

7. Later in 1983 our Stanford research group was the first to publish the identification of an enhancer element related to a heavy chain gene. It was not until several years later that results were published using cDNA clones in lymphoid cells: see e.g. Liu *et al* (1987) Gene 54, 33; Liu *et al* (1987) Proc. Natl. Acad. Sci. 84, 3439. These results using cDNA clones were only made possible by the discovery of enhancer elements and their incorporation into the cDNA construct. These enhancer elements were not known in the art in March 1983.

8. In section 6.4 of the Celltech Response, the work performed in Xenopus oocytes by Deacon, Valle I, Colman and Valle II is discussed. These prior art references disclosed that the mRNAs for both heavy and light chains are needed for the minimal expectation of synthesis and assembly. If the mRNA for only one chain were introduced, then it often was not secreted, depending upon the cells used. These results were consistent with earlier observations in certain mutant cell lines in which the presence of both light and heavy chains was required for secretion. On the basis of this work the skilled person understood in March 1983 that, to produce an assembled immunoglobulin in a mammalian host cell system, both the genes for the heavy and light chains would

have to be expressed in the same cell.

9. Consistent with the oocyte findings, the results of our Stanford research group reported in the 1983 PNAS paper showed that a host cell had to be transfected with the (genomic) genes for both heavy and light chains and that both chains had to be expressed in the suitable single host cell to obtain secreted functional antibody molecules. We expected to obtain such results in view of the prior art at the time. Accordingly, in March 1983, skilled persons attempting to obtain secretion of heterologous immunoglobulin molecules would have considered only a system in which the genetic material encoding both chains was present. Regardless of the differences between microinjecting mRNA into oocyte cytoplasm and transfecting host cells with immunoglobulin genes, as argued in section 6.4 of the Celltech Response, the oocyte work contributed to the antibody engineering art as examples of manipulating host cells to produce, assemble and secrete functional immunoglobulin molecules.

10. Sections 6.5.11 and 6.5.12 of the Celltech Response discuss our 1983 PNAS paper ("Oi"). The Patentee fails to note that the heterologous light chain gene used for transfection was a genomic clone. Genomic clones were also used by Rice and Baltimore and by Ochi. As discussed above, there are substantial differences between cDNA clones and genomic clones. Unlike cDNA sequences, genomic clones contain the necessary regulatory elements so that the encoded immunoglobulin chains can thereby be properly synthesized and processed by a suitable mammalian host cell.

Signed this ____ day of May 1995

Vernon T. Oi, Ph.D.

APPENDIX A

ScienceXchange

Curriculum Vitae and Selected Bibliography

Vernon Oi, Ph.D.
President, ScienceXchange

Education:

Stanford University Ph.D. 1981 Genetics
StanfordUniversity Postdoctoral Fellow 1982 Structural Biology

Experience/Training:

1995-present	President, ScienceXchange, Mountain View, California
1992-1995	Vice President, Helix Systems, Inc., Mountain View, California
1983-1992	Vice President Research Scientific Director Associate Scientific Director Group Leader Senior Scientist Becton Dickinson Immunocytometry Systems (BDIS), San Jose California
1982-1983	Research Associate, Department of Genetics, Stanford University

Patent:

August 22, 1989 US Patent No. 4859582: Stryer, L., A.N. Glazer, and V.T. Oi. 1989 Fluorescent Conjugates for Analysis of Molecules and Cells

Selected Publications:

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